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Steiner, U K

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Investment in defense and cost of predator-induced defense along a resource gradient

Ulrich K. Steiner

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Abstract An organism's investment in different traits to reduce predation is determined by the fitness benefit of the defense relative to the fitness costs associated with the allocation of time and resources to the defense. Inherent tradeoffs in time and resource allocation should result in differential investment in defense along a resource gradient, but competing models predict different patterns of investment. There are currently insufficient empirical data on changes in investment in defensive traits or their costs along resource gradients to differentiate between the competing allocation models. In this study, I exposed tadpoles to caged predators along a resource gradient in order to estimate investment in defense and costs of defense by assessing predator-induced plasticity. Induced defenses included increased tail depth, reduced feeding, and reduced swimming activity; costs associated with these defenses were reduced developmental rate, reduced growth, and reduced survival. At low resource availability, these costs predominately resulted in reduced survival, while at high resource availability the costs yielded a reduced developmental rate. Defensive traits responded strongly to predation risk, but did not respond to resource availability (with the exception of feeding activity), whereas traits construed as costs of defenses showed the opposite pattern. Therefore, defensive traits were highly sensitive to predation risk, while traits construed as costs of defense were highly sensitive to resource allocation tradeoffs. This difference in sensitivity between the two groups of traits may explain why the correlation between the expression of defensive traits and the expression of the associated defense costs was weak. Furthermore, my results indicate that genetic linkages and mechanistic integration of multiple defensive traits and their associated costs may constrain time and resource allocation in ways that are not addressed in existing models.

Keywords Adaptive plasticity · Phenotypic plasticity · *Rana temporaria* · Time allocation tradeoff · Trait integration

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U. K. Steiner
Institute of Zoology, University of Zürich,
8057 Zürich, Switzerland

U. K. Steiner (✉)
Biological Sciences, Herrin Labs,
Stanford University, Stanford,
CA 94304-5020, USA
e-mail: usteiner@stanford.edu

Introduction

Susceptibility to predation is a major determinant of fitness; therefore selection acts on traits that reduce predation. However, the optimal expression of defensive traits must also take into account any fitness costs associated with the investment in defense (Stearns 1992). There are competing conceptual models that predict a shift in the optimal allocation of time and resources to defense along a resource gradient, but the nature and direction of the shift vary greatly depending upon the model used.

The simple allocation model predicts increasing investment in defense with increasing resource availability (Harvell 1990; Tuomi et al. 1991; Werner and Anholt 1993). At low resource availability, all investment is made to maintain basic life functions and no investment in defense can be afforded. At high resource availability the maintenance of basic life functions is easily achieved and investment in defense is possible and beneficial.

In contrast to the simple allocation model, the defense–growth model predicts decreasing investment in defense with increasing resource availability (Myers and Bazely 1991). Growth is reduced at low resource availability, resulting in small individuals that are very vulnerable to predators and that spend more time at vulnerable stages (Arendt 1997). Individuals can grow quickly at high resource availability, allowing individuals to escape predation risk by either early metamorphosis or rapid attainment of a size beyond the reach of gape-limited predators (Kishida and Nishimura 2004). Therefore, defensive mechanisms are more important to slow-growing individuals, and investment in defense should be higher at low resource availability.

The growth differentiation model (Tuomi et al. 1991; Gomulkiewicz and Kirkpatrick 1992; Herms and Mattson 1992; Steiner and Pfeiffer 2007) combines the previous two models.This model follows the logic of the simple allocation model at low resource availability, whereas the model follows the logic of the defense–growth model at high resource availability. Therefore, investment in defense peaks at intermediate resources.

While allocations to defense are difficult to measure directly, the expression of defensive traits is clearly a function of underlying time and resource allocations and the predator-induced plasticity of these traits are reasonable approximations to investment (Van Buskirk 2000; Teplitsky et al. 2005). Predator-induced defenses have proved to be particularly useful in this regard (Tollrian and Harvell 1992). By exposing individuals to nonlethal predators, the expression of the full defensive response is exhibited without actually incurring predation. The difference in expression of defensive traits between induced and noninduced individuals, or the predator-induced plasticity in defensive traits (Tollrian and Harvell 1992), can be taken as a measure of investment in defense. Similarly, the predator-induced plasticity of nonadaptive traits (Agrawal et al. 2002; McPeek 2004) is a measure of the costs of defense. In this context, nonadaptive traits are traits that are related to fitness and respond to predation risk, but that do not decrease predation. As an example, an individual exposed to predators, in addition to expressing defensive traits, might reduce its growth rate (i.e., a nonadaptive trait). The induced reduction in growth rate can be viewed as the cost of allocating resources to defensive mechanisms. Note that nonadaptive responses are not maladaptive when seen from a predatory defense perspective, because the fitness benefit of the defense necessarily outweighs the costs.

Based on time and resource allocation arguments, a high investment in defense is associated with a high cost of defense. Therefore, investment in defense and costs of defense should be correlated, i.e., predator-induced plasticity of defensive traits should be correlated with predator-induced plasticity in nonadaptive traits.

Predator-induced defenses can be found in various morphological, behavioral, life-historical and physiological traits (Herms and Mattson 1992; Lima 1998). However, most studies of predator-induced defenses have not investigated different resource conditions, or they have used only two resource levels and could not detect nonlinear effects (Angilletta et al. 2003). Also, previous studies have not investigated investment in multiple defensive traits and their fitness costs in an integrated way (Van Buskirk 2000; Teplitsky et al. 2005).

The goal of this study was to investigate changes in investment and costs with various defensive traits and nonadaptive traits along a resource gradient. I used *Rana temporaria* tadpoles and one of their most common predators (the sit-and-wait predatory dragonfly larva *Aeshna cyanea*) as a model system. Tadpoles express multiple (adaptive) defenses, such as reduced swimming and feeding activity and an increase in tail depth (Skelly and Werner 1990; Van Buskirk and McCollum 2000). Costs of defenses are expressed in nonadaptive responses, such as reduced growth, reduced development and reduced survival not caused by predation (Skelly 1992; Anholt and Werner 1995; McCollum and Van Buskirk 1996; Anholt et al. 2000; Van Buskirk 2000, 2002; LaFiandra and Babbitt 2004). The defenses are known to reduce predation risk and the nonadaptive responses relate to reduced fitness but do not decrease predation (Van Buskirk and McCollum 2000; Altwegg and Reyer 2003).

I conducted an experiment in which I exposed tadpoles to a predator environment (with nonlethal caged predators) and a no-predator environment. I assessed the predator-induced responses along a resource gradient for three (adaptive) defensive traits (swimming, feeding, and tail depth) and three nonadaptive traits (body size, time to metamorphosis, and survival). I selected those traits because they largely respond independent of each other (Van Buskirk and McCollum 2000; Relyea 2002; Steiner 2005).

I did not have specific predictions for the responses of the different defensive traits, beside the general prediction that investment in defenses and cost of defenses should be correlated and investment in each defense trait should follow one of the models outlined above. To start with specific predictions would require a better understanding of the integration of multiple defensive traits and their associated costs. This study improves our understanding of the investment and correlated costs of defense and provides a basis for making predictions about genetic linkage and mechanistic integration of multiple traits.

Materials and methods

For the experiment I used *R. temporaria* tadpoles hatched from clutches collected from a population at an elevation of 1,159 m near Wildhaus, Switzerland. I reared tadpoles in 0.28 m^2 outdoor plastic pools filled with 80 l of water at the University of Zürich, Switzerland. At the outset on 4 May 2002 (day 1), I stocked 100 pools with 20 tadpoles (71 tadpoles/m^2), consisting of two individuals each from ten clutches. Stocked tadpoles were 4–5 days old (15 mg, stage 24–25, Gosner 1960). I covered the pools with a shade cloth to prevent predator invasions and metamorph escape. I moved tadpoles simultaneously to new pools when pool water started to get cloudy. This was done on days 19, 32, and 46. Water quality degraded only at the highest food level: the treatment with the highest survival. I terminated the experiment on day 54 (26 June 2002), when most tadpoles had reached metamorphosis.

Treatments

The experiment had two temperature treatments (warm and cold), two predator treatments (nonlethal predator and no-predator) and five food levels (resource availability), replicated in five complete randomized blocks (100 pools in total). The differences between the two temperature treatments (1.64 ± 0.05 °C) were not enough to manipulate growth rates as initially intended. I mention the temperature manipulation for integrity reasons and will not discuss the (lack of) temperature effect in detail. Details on the initial reasoning for manipulating temperature are available upon request.

Each pool contained one floating cage (~1 l in volume), which was either left empty or one final instar dragonfly larva (*A. cyanea*) was placed inside. I fed the dragonfly larvae 300 mg of *R. temporaria* tadpoles three times a week. This feeding schedule of the caged predators is known to induce a full defensive response in tadpoles exposed to the kairomone (the chemical cue released by the predator) (Van Buskirk and Arioli 2002). I rotated the dragonflies within the nonlethal predator treatment among the pools each time I fed them to equalize for possible differences among individual *Aeshna*. I rotated the cages among the no-predator pools to control for effects of disturbance.

The five resource availability levels were 1, 2, 4, 8, and 16% (16% equals ad libitum food) of tadpole body mass fed per day. Before each feeding event I calculated the amount of food by weighing two extra sets of twenty tadpoles for each resource availability level. After day 18 I measured the average wet weight of the experimental tadpoles in each treatment each week and based the amount of food on these averages, which allowed me to base the amount of dry food on the actual average wet weight of the experimental tadpoles in each treatment combination. I fed the tadpoles twice a week with a mixture of ground rabbit chow and fish food. I adjusted the amount of food in each pool to changes in tadpole densities, which were caused by metamorphosing individuals and mortality.

Sampling morphology, behavior, life history, and survival data

I measured morphology on each sampling day (18, 26, 33, 40, and 49) by randomly selecting five tadpoles from each pool and photographing them in lateral and ventral views. I present only the results from day 18 here. Results from day 26 were similar, but the data from later samples were unusable because tadpoles in high-resource treatments began entering metamorphic climax. I weighed the tadpoles (mass at day 18 is presented) and promptly returned them to the pool. I used image analysis software (Optimas 6.5, Media Cybernetics 1999) to measure tail depth and to estimate body size as the centroid size calculated from 26 landmarks positioned in three-dimensional space (Bookstein 1991, Electronic Supplementary Material S1). I obtained size-corrected tail depth by regressing the tail depth for all measured tadpoles against body size and the square of body size. Using size-corrected residuals can bias results (Darlington and Smulders 2001; Garcia-Berthou 2001; Freckleton 2002). Using an ANCOVA with body size and the square of body size did not alter the results (ANCOVA results are not reported). For ease of graphical display (Fig. 1), I used size-corrected residuals in all analyses of tail depth. I could not measure morphology and wet weight in two pools and in one pool respectively, due to technical problems.

I recorded behavioral data by instantaneously sampling the activity of the visible tadpoles as swimming,

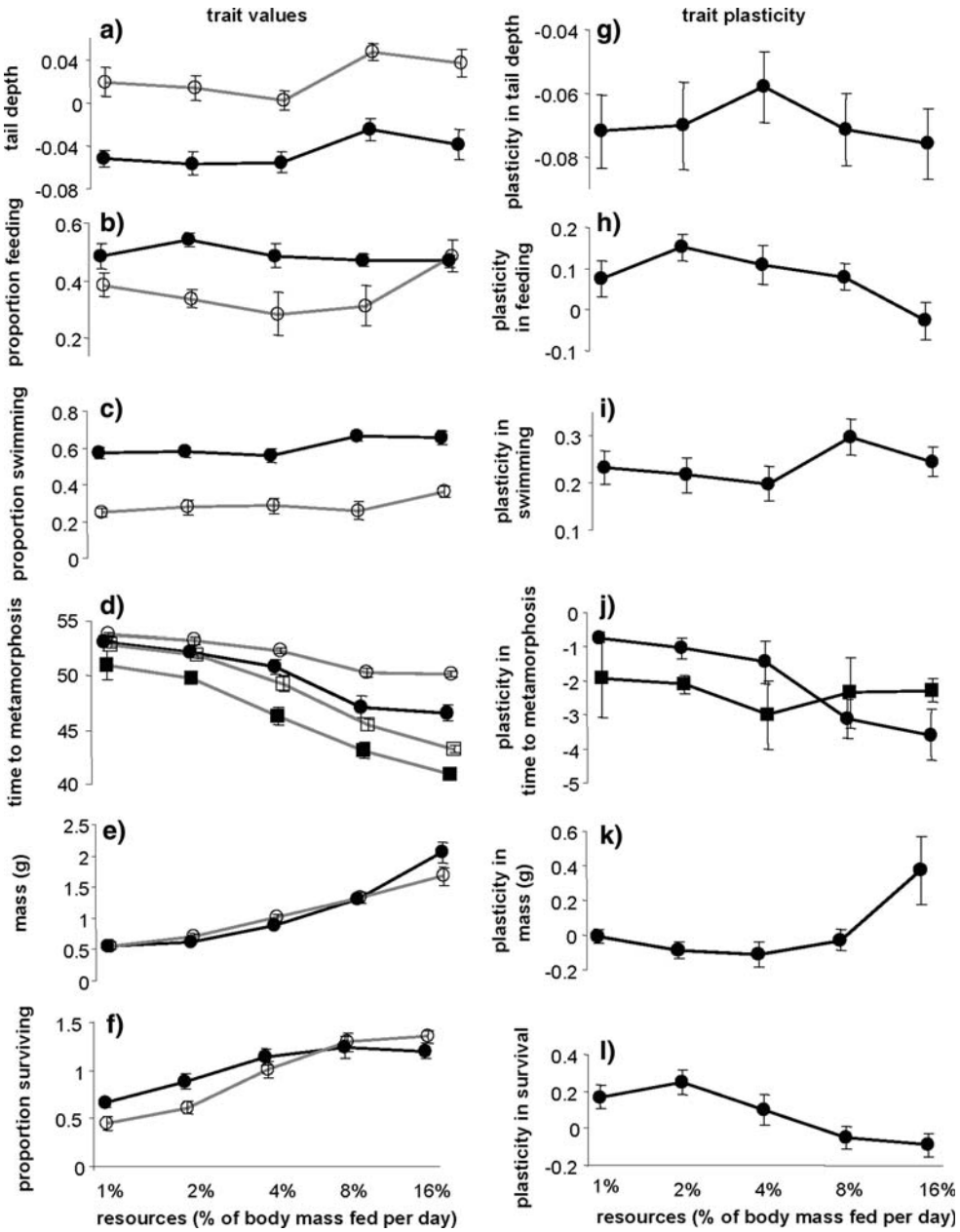

Fig. 1a–l **a–f** Expression of six traits in predator-naïve (*filled symbols*) and (nonlethal) predator-exposed (*open symbols*) *R. temporaria* tadpoles in response to increasing resource availability. For time to metamorphosis (**d**, **j**), *circles* indicate cold temperatures and *squares* warm temperatures. **g–l** Predator-induced plasticity in six traits in response to increasing resource availability. *Symbols* show means ± SE of five replicates. Predator-induced plasticity was measured as the difference in the trait expression between predator-naïve and predator-exposed tadpoles (note the negative values on the *y*-axis for **g**, **j**). Survival, feeding and swimming activity were arcsine-square-root-transformed (allowing values > 1). Tail depth values were body-size-corrected. For all traits shown in this figure, with the exception of time to metamorphosis, there was no significant effect of temperature on the response variables. I pooled results from the two temperature treatments in the graphs in order to simplify the graphical illustration of all of the traits except for time to metamorphosis

feeding or resting. Feeding behavior consists of scraping algae (mouth movement) at the pool walls and bottom, often accompanied by a characteristic tail waggle. Swimming was all other movements. I recorded the data by visiting each pool four times over a three and a half hour period on day 22, one day after I fed the dragonflies and the same day I fed the tadpoles. Most tadpoles were visible—only 4.6% were hiding.

Given that all tadpoles entered the experiment at the same age and date, I used time to metamorphosis as a measure of developmental rate. I removed tadpoles that reached the four-emerged-limbs stage (Gosner stage 42) from the pools and kept them in tilted boxes with little water until the tail was absorbed (stage 46). I noted the date when metamorphosis was completed (Gosner stage 46). I checked the pools and boxes for metamorphs at least every second day. I noted tadpoles (17.7%) that did not reach the four-emerged-limbs stage by the end of the experiment (day 54) as metamorphosed on day 55. This is a conservative method of analysis, because those 17.7% of the tadpoles were noted as metamorphosed on (theoretically) the earliest possible date.

I noted tadpoles that reached the four-emerged-limbs stage (Gosner stage 42) by day 54 as being survivors. Nonsurviving tadpoles were tadpoles that disappeared or died before day 54, and those 17.7% that did not reach the four-emerged-limbs stage by day 54. Tadpoles

that do not metamorphose that late in the season have low survival probabilities (Altwegg and Reyer 2003). To ascertain that my definition of survival for the tadpoles that did not reach the four-emerged-limbs stage had no substantial effect on the results, I reanalyzed the survival data while excluding these tadpoles. Results changed only slightly (mixed model as described in statistical analyses, with survival as response variable: logarithm of food $F_{(1,88)} = 32.55$, $P < 0.0001$; predation $F_{(1,88)} = 11.38$, $P = 0.0011$; logarithm of food by predation interaction $F_{(1,88)} = 14.25$, $P = 0.0003$).

Statistical analyses

I performed two tests for each trait. First, I tested the overall effects of predators, temperature and food and possible interactions on the six traits with a mixed model (proc mixed, type III SS, SAS 9.1, SAS Institute 2002) including block as a random factor, predator and temperature as categorical fixed factors, and the logarithm of food levels as a continuous fixed factor. I defined each pool as an independent unit. For all traits I used the pool means in the analysis. I arcsine-square-root-transformed survival and behavioral ratios for all analyses (allowing values > 1, Fig. 1f). I accounted for multiple testing (six traits) with Bonferroni corrections. I used the logarithm of food availability in all analyses, because I believe that doubling the amount of food is more biologically meaningful to an individual than increasing the amount of food by a specified number of units (mg). I describe only the main effects of food and predators for this first test, because interactions between food and predators are investigated in the second test. For temperature, I only describe significant main effects and significant interactions between temperature and food. All interactions between temperature and predators, and temperature, predators and food were nonsignificant (see Table 1).

The second test describes the shape of the predator-induced plasticity along the resource gradient. Predator-induced plasticity was the absolute difference between the no-predator and nonlethal predator treatments for the different traits (calculated for each treatment combination within each block). I used absolute plasticity values to get a direct scale unit with the exception of tail depth, which was corrected for body size. No trait response curve was fundamentally altered, nor does the interpretation of the results change when I analyze relative values. To describe the shape of the predator-induced plasticity along the resource availability gradient, I used model selection, based on Akaike's Information Criterion of small samples (AICc), on three candidate models. I assessed the support for each model for each trait separately. The three candidate models included (a) an intercept-only model, which describes no change in predator-induced plasticity along the resource gradient (control model), (b) a linear term for the resource availability effect (simple allocation model and defense–growth model), and (c) a linear and quadratic term for the resource availability treatment (growth differentiation model). I calculated the Akaike weight and evidence ratio to determine how much better the best fitting model was supported in comparison to the other models (Burnham and Anderson 2002). I used model selection and not a second-order model because I was interested in the overall support for the model and did not want to

Table 1 Relationship between predator exposure and temperature differences along a resource gradient (logarithm of food) for six different traits

	Tail depth $F_{1,86}$	Feeding activity $F_{1,88}$	Swimming activity $F_{1,88}$	Time to metamorphosis $F_{1,88}$	Mass $F_{1,87}$	Survival $F_{1,88}$
Block	0	0.49	0	0.79	1.08	0
Predator	**33.93***	**13.9***	**74.5***	**8.58***	2.47	**11.87***
Temperature	1.3	1.03	0.56	**6.81**	0.06	1.7
Predator × temperature	0.3	0.77	0	3.85	0.35	0.03
log Food	**7.26**	0.13	**9.58***	**511.13***	**318.3***	**121.66***
log Food × predator	0.04	1.89	0.08	**6.03**	**5.16**	**9.22***
log Food × temperature	2.3	0.17	0.41	**46.67***	3.78	1.81
log Food × predator × temperature	0.18	0.19	0.61	3.68	1.06	0.09

Mixed effect models are presented with block used as a random factor. The interaction between the logarithm of food and predator exposure is investigated in more detail in the results presented in Table 2. Reported degrees of freedom account for all tests except the tests for block effects (random factor)

F values in boldface indicate significance prior to Bonferroni corrections ($P < 0.05$)

* Significance after Bonferroni corrections for six tests ($P < 0.0083$)

test the significance of each single parameter in the model.

Results

Predator-exposed tadpoles had 11.6% deeper tails than predator-naïve tadpoles (Fig. 1a, Table 1). Predator-induced plasticity in tail depth, the difference between predator-exposed and predator-naïve tadpoles, which I used to measure investment in defense, did not change with increasing resource availability (Fig. 1g, Table 2).

Behavior responded strongly to predators (Table 1), but only swimming activity changed with resource availability disregarding predation risk (Fig. 1b, c, Table 1). Feeding activity decreased in response to predator exposure by 34%. Swimming activity decreased in response to predators by 72%, and with increasing resource availability it increased by 39%. The predator-induced plasticity in feeding activity was greatest at intermediate food levels and was therefore best explained by a curvilinear relationship (Fig. 1h, Table 2), while the predator-induced plasticity in swimming activity did not change with increasing resource availability and was best explained by an intercept-only model (Fig. 1i, Table 2).

Time to metamorphosis was affected by resource availability and predation risk (Fig. 1d, Table 1). At high resource availability, tadpoles metamorphosed about eight days earlier than at low resource availability and predator-naïve tadpoles metamorphosed about two days earlier than predator-exposed tadpoles. Temperature affected the time to metamorphosis only at high resource availability but not at low resource availability (Fig. 1d, Table 1). Predator-induced plasticity in time to metamorphosis increased along the resource gradient and was best described by a linear relationship (Fig. 1j, note negative values; Table 2).

Tadpole mass responded strongly to resource availability (Fig. 1e, Table 1). At high resource availability, tadpoles weighed three times more than at low resource availability. Predator exposure affected tadpole mass only at the highest resource availability, with predator-naïve tadpoles showing higher masses (Fig. 1e). The predator-induced plasticity in body mass along the resource gradient was best explained by a curvilinear relationship (Table 2).

Survival increased with increasing resource availability and was reduced under predation risk (Fig. 1f, Table 1). At high resource availability survival was almost three times higher than at low resource availability, and 11% more (nonlethal) predator-exposed tadpoles died than predator-naïve ones. The survival advantage of predator-naïve tadpoles ("predator-induced plasticity in survival") decreased with increasing resource availability (Fig. 1l) and was best explained by a linear relationship (Table 2).

Discussion

The tadpoles in the experiment exhibited the previously described responses to resource availability and predation risk (Skelly and Werner 1990; Skelly 1992; Anholt and Werner 1995; McCollum and VanBuskirk 1996; Werner and Anholt 1996; Laurila et al. 1998; Van Buskirk and Yurewicz 1998; Laurila and Kujasalo 1999; Anholt et al. 2000; Van Buskirk 2000; Peacor 2002; LaFiandra and Babbitt 2004; Relyea 2004). Under limited resource availability, they showed reduced mass, reduced developmental rate, and had

Table 2 Model selection procedure used to evaluate support for three candidate models, including intercept-only, linear (intercept + log food) and nonlinear (intercept + log food + log food^2) relationships between predator-induced plasticity and resource availability for six different traits

	Intercept only		Intercept ± log food		Intercept ± log food ± log food^2		Evidence ratio 1	Evidence ratio 2
	AICc	Akaike weight	AICc	Akaike weight	AICc	Akaike weight		
Tail depth	**−181.7**	**0.9872**	−177.2	0.0110	−175.4	0.0018	90.0	544.6
Feeding	−50.4	0.0259	−50.4	0.0259	**−54.0**	**0.9482**	36.6	36.6
Swimming	**−71.3**	**0.9786**	−67.4	0.0198	−64.9	0.0016	49.4	601.8
Time to metamorphosis	198.5	0.0003	**190.5**	**0.9238**	193.0	0.0758	12.2	2980.9
Mass	45.3	0.0001	42.8	0.0012	**36.1**	**0.9987**	812.4	9897.1
Survival	7.2	0.0003	**−0.8**	**0.8453**	0.9	0.1544	5.5	2980.9

The AICc and Akaike weight of the best supported model is boldfaced. Evidence ratio 1 describes how much better the best fitting model is supported in comparison to the second best model, and evidence ratio 2 describes how much better the best fitting model is supported in comparison with the least supported candidate model

lower survival. Under predation risk, defenses were expressed by increased tail depth and reduced feeding and swimming activity. Costs of defense were mostly expressed by a reduced developmental rate and reduced survival.

The costs of defense shifted along the resource gradient. At low resource availability, defense costs did not result in a reduction in development rate or reduced mass, but the consequence for defense was reduced survival. Reduced survival probabilities in response to (nonlethal) predators at low resources have been found in other studies (Peacor 2002). At high resource availability, the costs of defense were a reduction in the development rate without a reduction in survival, in agreement with previous work (Skelly 1992; LaFiandra and Babbitt 2004). Costs of defense were also expressed by reduced mass in predator-exposed tadpoles, but only at the highest resource level. Most previous studies were not designed to detect shifts in costs along resource gradients, because they either did not manipulate resources or did not investigate costs for multiple traits. The reported shift in costs along the resource gradient indicates that survival and development are mechanically or genetically linked. The shift in costs is likely linked to shifts in the time and resource allocation tradeoff along the resource gradient. For low resources, the time to reach metamorphosis before the end of the growing season is very constrained (Steiner and Pfeiffer 2007). Many resources need to be directed to reaching a threshold developmental rate and no resources are available to pay for the cost of defense. The consequence of the cost of defense is then reduced survival. Reduced survival is most likely a cumulative result of numerous effect pathways and reduced allocation to maintenance and should be closely linked to resource allocation tradeoffs. Under high resources, time allocation tradeoff are relaxed, because it is easy to reach metamorphosis before the end of the growing season. Therefore, the costs of defense are a reduction in development rate and not a reduction in allocation to maintenance, resulting in similar rates of survival between predator-exposed and unexposed tadpoles.

I expected a strong correlation between investment in defense and cost of defense. Defense and costs of defense were expressed at each resource availability level. However, in contrast to my expectation, investment in defense and cost of defense were not strongly correlated. Predation risk had a more severe effect on defensive traits than resource availability, as shown by strong predator-induced plasticity but only weak resource-induced plasticity (responses to resource availability). The opposite pattern was found for nonadaptive traits. The enhanced resource-induced plasticity in the nonadaptive traits indicates that they incur a stricter resource allocation tradeoff rule, while defensive traits were strictly ruled by the predation risk. This difference in dominance explains why there was no strong correlation between investment in defense and costs of defense.

Defensive traits have evolved in response to predation risk and should therefore act more specifically in response to predation risk, as found in my study. Nonadaptive traits, traits that are related to fitness but do not reduce predation, should have evolved in response to many environmental factors, including resource availability. Selection should act to reduce cost of defense, i.e., weak responses to predation risk in nonadaptive traits but strong responses to predation risk in defensive traits are selected for, resulting in the observed pattern of differences in dominance. Defensive traits respond more specifically to predation risk compared to nonadaptive traits and show less variability in their response (less interactive effects; Tables 1, 2). One could conclude that defensive traits show less variability than nonadaptive traits. However, there is a limitation to this conclusion, because most defenses investigated in zoological systems are behavioral or morphological trait responses, whereas traits where costs of defense are expressed are often life history traits. Hence, we need systems with life history defensive traits and morphological and behavioral nonadaptive traits, e.g., systems where development time is reduced under predation risk.

I expected, in accordance with the models outlined in the Introduction, there to be an interaction between resource allocation and predation risk in defensive traits. The lack of this interaction in most defensive traits might be due to the high level of predation risk in the experiment. If predation risk dominates over resource availability, individuals should express their maximal defense regardless of the resource availability and the costs of defense. The maximal defense is limited by the maximal phenotypic plasticity, which is genetically determined. I think that defense in tail depth and swimming activity was expressed at maximum across the resource gradient, which explains why none of the models outlined in the introduction was supported by these traits. Some support for this explanation comes from studies that show that defense is limited and levels off when predation risk continues to increase above a certain threshold (Van Buskirk and Arioli 2002; Relyea 2004; Teplitsky et al. 2005). Support for my findings and disagreement with the theoretical models comes from studies that manipulated resource availability. All of them failed to find a

significant interaction between food level and predator effect in defensive traits (Skelly and Werner 1990; Anholt and Werner 1995; Laurila and Kujasalo 1999; Anholt et al. 2000; Peacor 2002; LaFiandra and Babbitt 2004). All of these studies used high levels of predation risk. However, studies which manipulated tadpole densities found an interaction between density and predator effect in defenses, as proposed by the simple allocation model (Werner and Anholt 1993; Relyea 2004). The difference in results between the two groups of studies suggests that competition effects cannot be viewed as equivalent to resource manipulation effects, because conspecific densities might change the abundance of cues in the environment, change the relative predation risk, or might affect resource availabilities in unexpected ways (Peacor 2002, 2003).

However, one defense trait was affected by resource levels, suggesting that the dominance of the predation risk in defensive traits was not absolute. Feeding activity agreed in its investment in defense (predator-induced plasticity) with one of the theoretical models, the growth differentiation model. At low resource availability the feeding activity was dominated by acquisition of the scarce resources and no strong response to predation risk was expressed, which has been described before (Werner and Anholt 1996; Van Buskirk and Yurewicz 1998; Relyea 2004). At high resource availability the strategy employed by predator induced-tadpoles was to spend as much time feeding as the predator-naïve tadpoles. One explanation could be that at high resource availability tadpoles escaped predation by reaching a size threshold as outlined in the simple allocation model. However, we would expect a similar reduction in investment in defense for the swimming activity. Predator-exposed tadpoles might also be willing to take a higher risk when foraging at high resource availability for unknown reasons. If we accept that defense is expressed by reduced feeding activity and that feeding activity is optimized to maximize fitness, we have to conclude that a reduction in feeding activity under predation risk is only beneficial at intermediate resource availability, i.e., investment in defense is only made at intermediate resource availability. I expected that high investment in defense at intermediate resources would evoke high costs at intermediate resources, which was not the case. This shows that defense and its costs are not closely linked.

Empirical data on interactions between resource allocation and predation risk in defensive traits in other systems are rare. A peak investment in defense at intermediate resources has been described for *Daphnia* head length (Barry 1995). The best empirical data comes from plant–herbivore systems and induced chemical defenses, but in many of these studies it is difficult to distinguish between defense (adaptive responses) and cost of defense (nonadaptive responses) (Karban and Baldwin 1997; Agrawal et al. 2002). We need studies that investigate effects across various resource levels and various predation risk levels.

The different environments experienced by predator-induced and predator-naïve tadpoles in the experiment likely cover the extremes observed in nature (Van Buskirk and Arioli 2002). We know that predator densities vary substantially in natural ponds (e.g., low predator densities in temporal ponds and high predator densities in permanent ponds). We also know that tadpole survival in natural ponds is low (5–7%) and we assume that predation is the major cause (Riis 1991). However, accurately estimating real predation rates or quantifying chemical cues (kairomones) in nature is difficult (Van Buskirk 2005; Van Buskirk and Arioli 2005). The variation in resource availability used in the experiment is also likely to be found in nature. There are natural ponds with ad libitum food. When we consider that some *R. temporaria* lay their clutches of thousands of eggs in temperate ruts, it becomes clear that resource availability in nature can be very low. Conducting experiments at the extremes (high predation risk, ad libitum food) might be problematic and can reveal different defense patterns than observed in experiments performed under intermediate conditions (Steiner and Pfeiffer 2007).

Not all studies exploring the costs of defense in tadpoles and other systems are consistent in their findings. Opposite patterns in growth rates, size at metamorphosis, and survival in response to (nonlethal) predators have been found (Werner and Anholt 1996; DeWitt et al. 1999; Van Buskirk 2002; Benard 2004; Hoverman et al. 2005). Some of this variation might be due to different adaptations to various predator types and different defense strategies of prey species (Laurila et al. 1998; Hoverman et al. 2005). Some variation might also be explained by differences in resource availability. If only one of the nonadaptive traits had been assessed in this study the conclusion drawn would have changed. There would have been reduced (no) costs of defense at low or high resource availabilities respectively, despite equal amount of defenses across the whole range of the resource gradient. It is important to assess the costs and benefits of defense on multiple potentially interacting traits.

The predicted correlation between defense and cost of defense was not demonstrated by the results from this study. For a better understanding of the origin of the discrepancy between theories and empirical data,

we need a better knowledge of the effect pathways that link defense and costs of defense. For instance, the widely assumed link between feeding activity, resource acquisition and conversion of acquired food into body mass (growth) has been shown to be unclear in tadpoles and a number of damselfly larvae (McPeek 2004; Steiner 2005). An improved knowledge of effect pathways would allow us to develop allocation models that more closely approach empirical data, and which include intrinsic costs that can explain the link between defenses and associated costs (Yearsley et al. 2002). A better understanding of mechanistic integration and genetic linkage of multiple traits is important when attempting to make predictions about adaptation to various environments, which have implications for population dynamics, adaptation to changing environments and community dynamics. My study shows that non-adaptive traits, where costs of defenses are expressed, are mechanistically or genetically linked (shift in cost along the resource gradient), but that defenses and their costs are largely independent of each other. We currently lack a good understanding of the evolution of complex traits, and more studies on (predator-) induced plasticity in various systems along environmental gradients such as resource and predation risk, spanning multiple behavioral, physiological, morphological, and life-historical traits, would likely fill this gap.

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